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Mapping of HLA genes using pulsed-field gradient electrophoresis

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The technique of pulsed-field gradient electrophoresis (PFGE) allows the determination of gene linkage relationships since DNA fragments up to 2 Mb can be separated. PFGE was employed to study linkage of class I, II and III genes belonging to the human major histocompatibility (HLA) complex. The results establish that the class II DOβ and DZα genes are linked with the DP subregion, centromeric to the DQ/DX-DR-C4 chromosomal segment, and allow us to estimate the minimal length of the entire HLA complex.

Pulsed-field gradient electrophoresis *Gene linkage* *HLA complex*

1. INTRODUCTION

The HLA complex is situated on the short arm of chromosome 6 in the distal part of the 6p21.3 band [1,2]. It consists of three regions containing the class I, II and III genes. Family studies have established that the order of these regions on 6p is centromere – class II – class III – class I [1]. The HLA class II genes are clustered in at least four subregions: HLA-DP, -DQ, -DX and -DR, each containing a minimum of one α and one β gene (review [3]). Two more genes, DOβ [4] and DZα [5] are also in the class II region. With the exception of the HLA-DP loci, which map centromeric to DQ and DR, the arrangement of genes in the class II region is unknown [1]. PFGE has been successfully applied, e.g. to analyze amplicons in mammalian DNA [6] and this prompted us to localize genes within the HLA complex using this technique.

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Abbreviations: HLA complex, human major histocompatibility complex; PFGE, pulsed-field gradient electrophoresis; kb(Mb), kilo(mega)base pair(s)

2. MATERIALS AND METHODS

To avoid interpretative difficulties which might arise from haplotype-specific restriction fragment length polymorphisms, mutant human cell lines with monosomy 6 or HLA hemizygosity were employed. All mutants were derived from wild-type BJAB-B95.8.6 lymphoma cells with the HLA haplotypes A1, Cw4, B35, Bw6, DR5, DRw52, DQw3, DPw4 and A2, C-, B13, Bw4, DRw6, DRw52, DQw1, DPw2 ([2,7] and unpublished). Mutant BM11.1 has lost genes of the A2 haplotype with the possible exception of DP and shows an interstitial deletion on the short arm of one chromosome 6 [2]. BM19.7 is a cloned subline of BM19 [7] and has lost the chromosome 6 bearing the A1 haplotype. BM28.7 was produced from irradiated wild-type cells using selection against HLA-A2 with the monoclonal antibody TÜ 160 [8] and complement. Like BM19.7, BM28.7 also exhibits monosomy 6 (Fonatsch, C., unpublished), but with retention of the A1 haplotype. The PFGE system developed by Schwartz and Cantor was modified as described by Johnson and Borst [9].

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The preparation of digested mammalian DNA was as described in [6]. The chromosomal DNA, embedded in agarose to avoid shear, was digested with the following infrequently cutting restriction endonucleases: *Mlu*I, *Nae*I, *Not*I, *Nru*I, *Pae*R7, *Pvu*I, *Sfi*I, *Sma*I and *Xho*I. After PFGE, blots were hybridized under stringent conditions [10] with probes for class I (pi6) [11], C4 [12], DRα [13], DRβ [14], DQβ [3], DQα [3], DOβ [4], DZα [5], DPα [3] and DPβ [3] genes.

3. RESULTS AND DISCUSSION

Since results with the restrictive enzyme *Not*I were most informative, only such data will be reported here, although experiments with other enzymes support the conclusions. Hybridization of C4, DRα and DRβ probes with *Not*I-digested, PFGE-separated DNA from wild-type and complementary loss mutants is shown in fig.1. Irrespective of the haplotype analyzed, all probes seem to hybridize to the same fragment with an approximate length of 1 Mb. The wild-type DNA in fig.1A and B appears to have been only partially digested, and the C4 and DRα genes are still on the same fragment. This is of course expected when C4 and DRα are situated on the same and not on two different fragments of similar sizes. Hybridization with C4, DRα and DRβ probes of blotted BM19.7 DNA separated in a single PFGE run (fig.2A) gave the expected result. DQα/β and the crosshybridizing DXα/β genes were also present in the 1 Mb fragment (fig.2B). However, DOβ, DZα and DPα/β gene probes hybridized to one distinct fragment of 600 kb length (fig.2B). Various pulse frequencies during PFGE gave results leading to the same conclusion.

Hybridization of *Not*I digested, PFGE-separated DNA from BM11.1 with a class I probe revealed two bands with approximate sizes of 0.8 and 1.2 Mb (fig.3). The same blot was also hybridized with C4 and DRα probes respectively, and the expected 1 Mb band was observed. We conclude that all class I loci must be present on two chromosomal fragments which are not identical with those containing the class II and C4 genes. The linkage of the DOβ and DZα genes with the DP subregion clearly maps them centromeric to the DQ/DX/DR genes.

Our results do not allow conclusions to be drawn

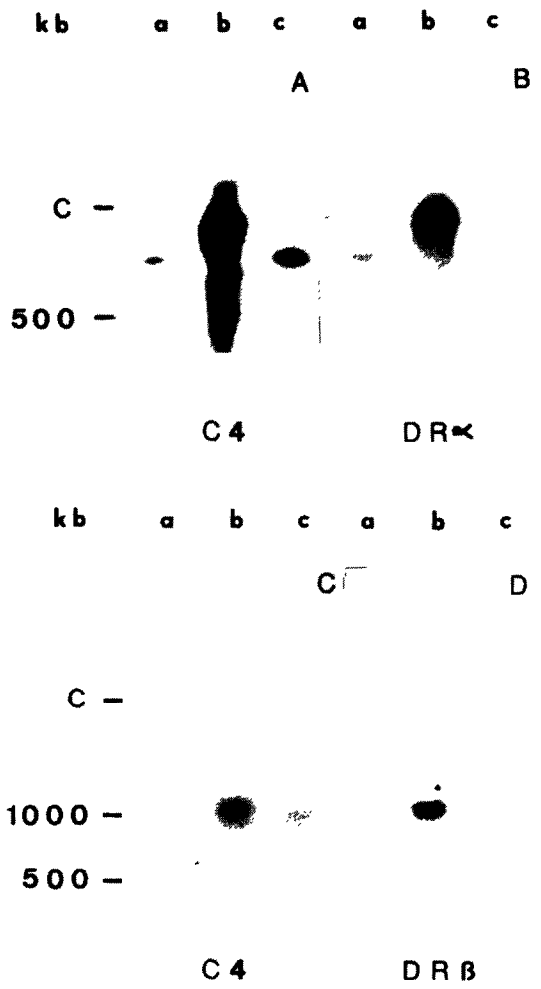
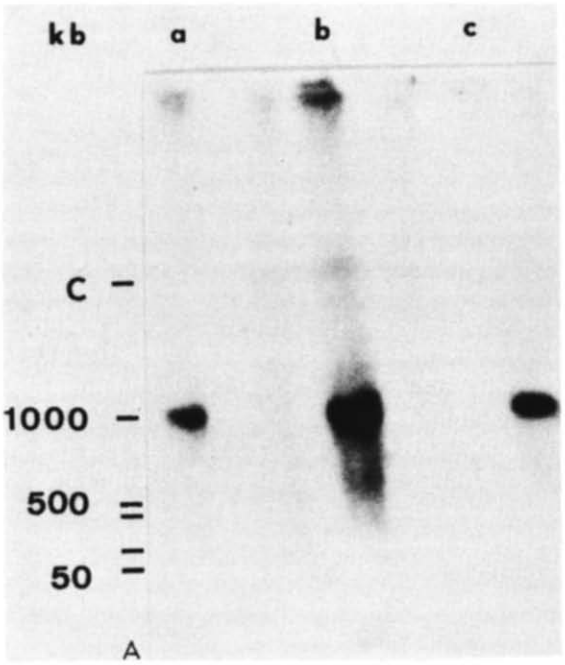


Fig.1. (A,B) Demonstration of C4 and DRα linkage. BM19.7 (lane a), BJAB-95.8.6 (b) and BM28.7 (c) cells were digested with *Not*I. A 120 s pulse was applied for PFGE and the blot was hybridized with a C4 probe recognizing C4A and C4B, and subsequently with a DRα probe. Approximate lengths (in kb) of λ concatemers are given on the left. C indicates the compression zone in PFGE. The DNA of wild-type cells was only partially digested. (C,D) Demonstration of C4 and DRβ linkage. Lanes a–c as in A,B. A 180 s pulse was applied for PFGE. The blot was hybridized with the C4 probe and subsequently with the DRβ probe. Approximate length (in kb) of *Trypanosoma brucei* (strain 427) chromosome markers is indicated.

regarding the accurate physical distance between the class II gene clusters, mainly because the sizes of DNA fragments larger than 700 kb are extrapolated and therefore only tentative in PFGE

[9]. However, addition of the individual *Not*I restriction fragments makes it possible to estimate the approximate minimal length of the entire HLA region to be 2.5 Mb.

It appears therefore that PFGE clearly has the potential to close the gap between conventional human cytogenetics and molecular biological methods. With the DNA cloning procedures available today, the production of a restriction map of an entire chromosome using PFGE does not seem to be an impossible task. Chromosome 6 may be a suitable candidate for such work because



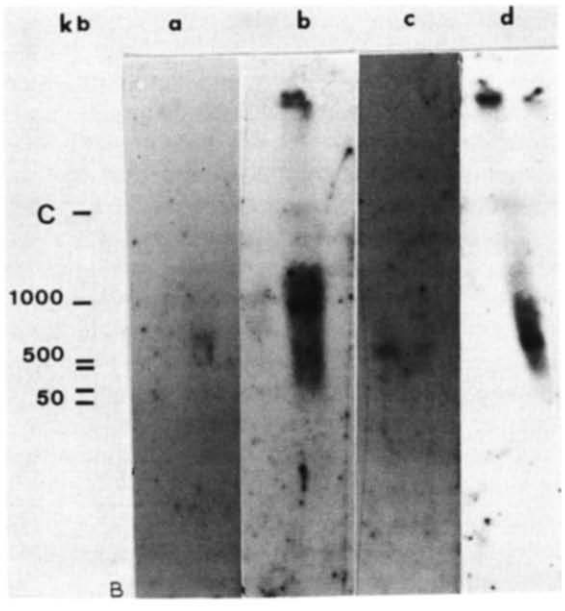


Fig.2. (A) Demonstration of C4-DRα-DRβ linkage in the A2 haplotype. BM19.7 cells were digested with *Not*I and the DNA separated in a 180 s pulse PFGE. The blot was cut in strips, each containing one of three parallel lanes, which were then hybridized with C4 (a), DRα (b) and DRβ (c). Length markers as in fig.1C. (B) Demonstration of DR/DQ and DOβ-DZα-DP linkage. Washed strips from A were rehybridized with a DQβ probe lane (b). DQα also hybridized on the same fragment (not shown) which should contain the cross-hybridizing DXα/DXβ genes as well. This 1 Mb fragment is identical to the one carrying C4-DRα/DRβ genes showing the linkage of the DQ/DX to the DR subregion. Lane a was hybridized with DOβ, lane c with DZα and lane d with DPα. All probes hybridized with a 600 kb fragment as well as DPβ (not shown).

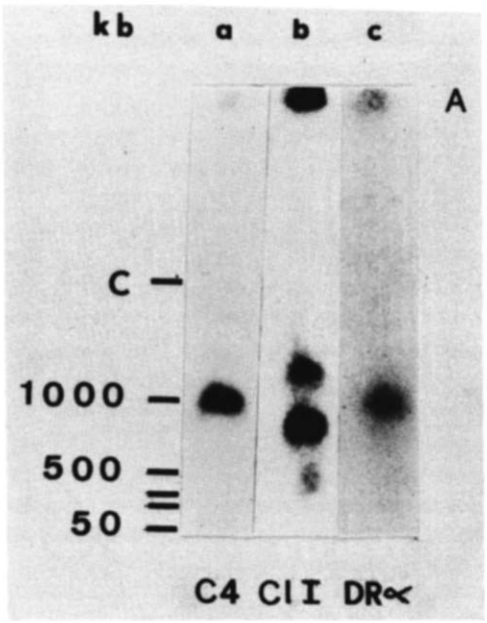


Fig.3. HLA class I and class II genes are not on the same *Not*I fragment. BM11.1 cells were digested with *Not*I and separated in a 180 s pulse PFGE. The blot was hybridized first with C4 (a), then with class I (b) and finally with the DRα probe (c). Length markers as in fig.1C.

of the existence of complementary monosomy 6 ([7]; this paper) and several interstitial deletion mutant cell lines [2,15,16].

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